

FIGURE 1

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)  
*Escherichia coli* B Phytase Sequence

1  
 ATG AAA GCG ATC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG  
 Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro  
 CAA TTT GCA TTC GCT CAG AGT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG  
 Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Gln Ser Val Val  
 ATT GTC AGT CGT CAT GGT GTG CGT GGT CCA ACC AAG GCG ACC GAA CTG ATG  
 Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met  
 CAG GAT GTC ATC CCA GAG GCA TGG CCA ACC TGG CTG GTA AAA CTG GCT TGG  
 Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp  
 CTG AAA CCG CCG GGT GGT GAG CTA ATC GCG TAT CTC GGA CAT TAC CAA CCG  
 Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg  
 CAG CTT CTG GTA GCG GAG GGA TTG CTG GCG AAA AAG GCG TCG CCG CAG TCT  
 Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser  
 GGT CAG GTC GCG ATT ATT GCT GAT GTC GAG CAG CGT ACC CTT AAA ACA GCG  
 Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly  
 GAA CCG TTC GCG GCG GCG GCG GGA GCT GAG TGT GCA ATA ACC GTA CAT ACC  
 Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr  
 CAG CCA GAT AAG TCG AAT CCG GAT GAG TTA TTT AAT CCG GCA AAA ACT GCG  
 Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn His Leu Lys Thr Gly  
 GGT TCG GAA ATG GAT AAC GCG AAC GTG AAT GAG CCG ATC CTC ACG AGG GCA  
 Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala  
 GGA GCG GCA ATT GCT GAT TTT ACC GCG CAT CCG GAA ACG GCG TTT CCG GAA  
 Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu  
 CTG GAA GCG CTG CTT AAT TTT CCG GAA TCA AAC CTC TCG CTT AAA CCG GAG  
 Leu Glu Arg Val Leu Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu  
 AAA TAG GAC GAA AGC TGT TCA TTA ATA CAG CCA TTA GAA TCG GAA CTC AAG  
 Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys  
 GTG AGT GCG GAC AAT CTC TTA TTA AAC GGT CCG GTA AAC CTC GCA TCA ATG  
 Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met  
 CTG ACC GAG ATA TTT CTC CTG GAA GAA GCA CAG GGA AAG CCG GAG CCG GCG  
 Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly  
 TGG GGA ACG ATC ACC GAT TCA CAG CAG TGG AAC ACC TTG GTA AGT TTG CAT  
 Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His  
 AAC GCG CAA TTT TAT TTG CTA CAA CCG ACG CCA GAG ATT GCG CCG ACG TCG  
 Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg  
 GCG ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCG CAT CCA CCG  
 Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro



**FIGURE 2**  
**pH/Temperature Profile and Stability**

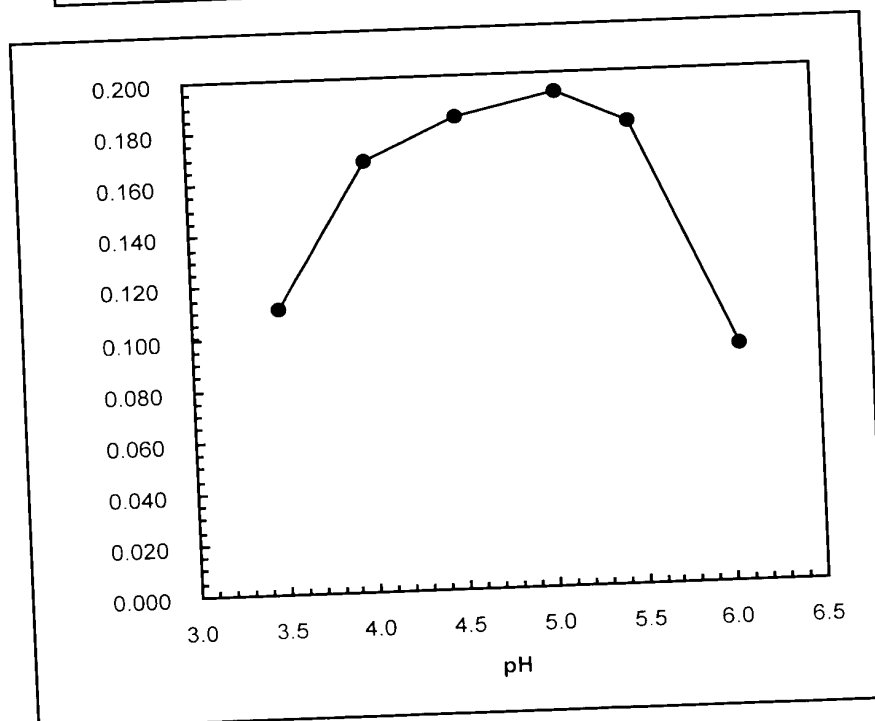
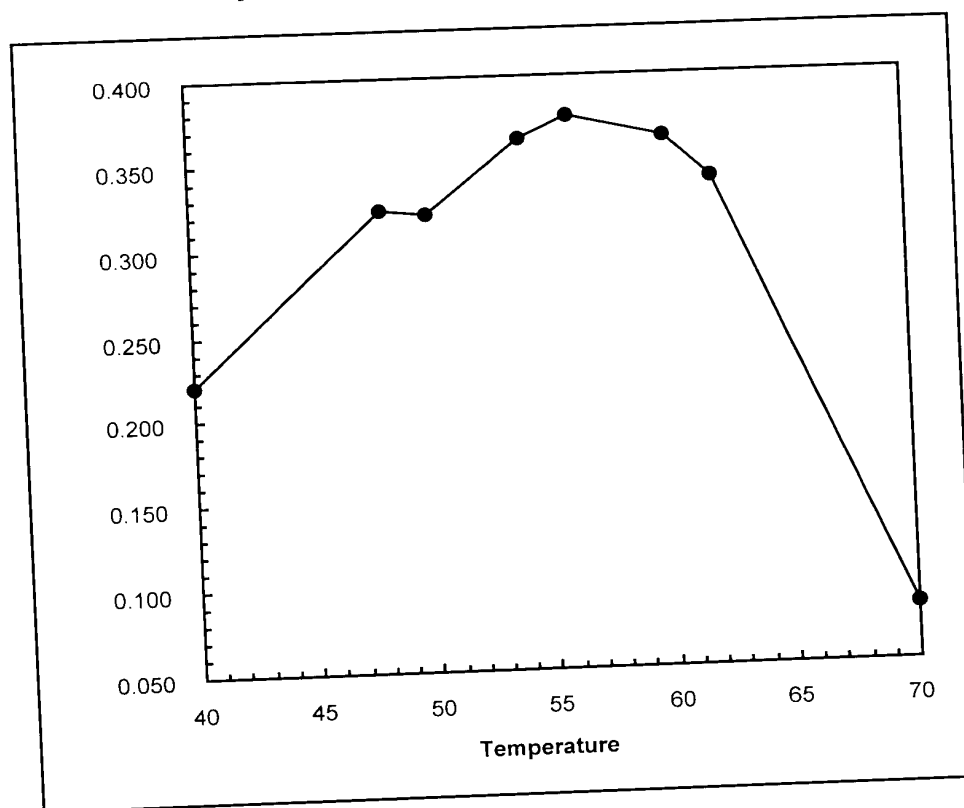


Figure 3:

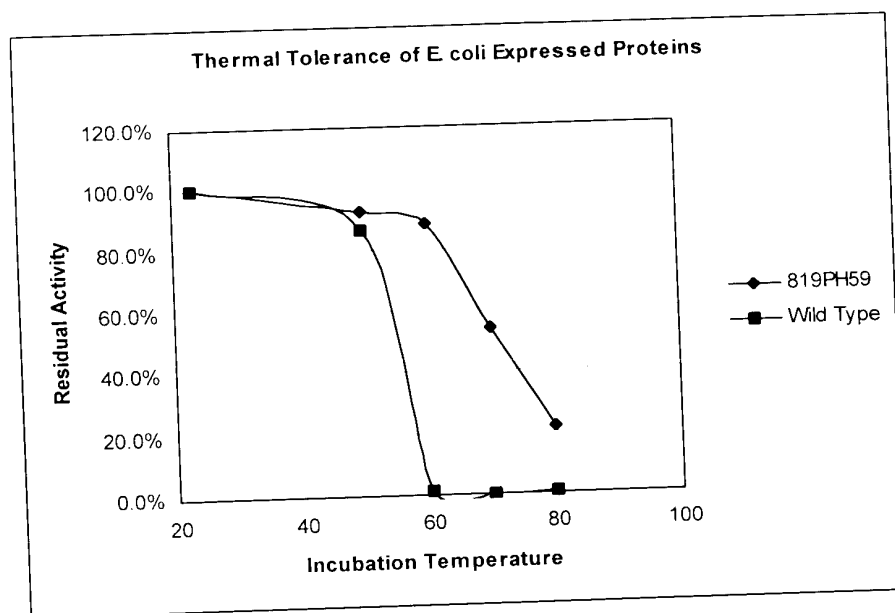


Figure 4

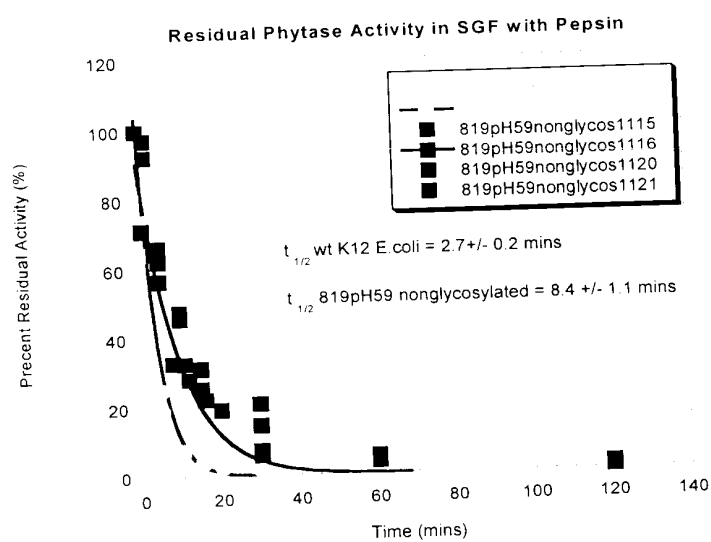


Figure 5:

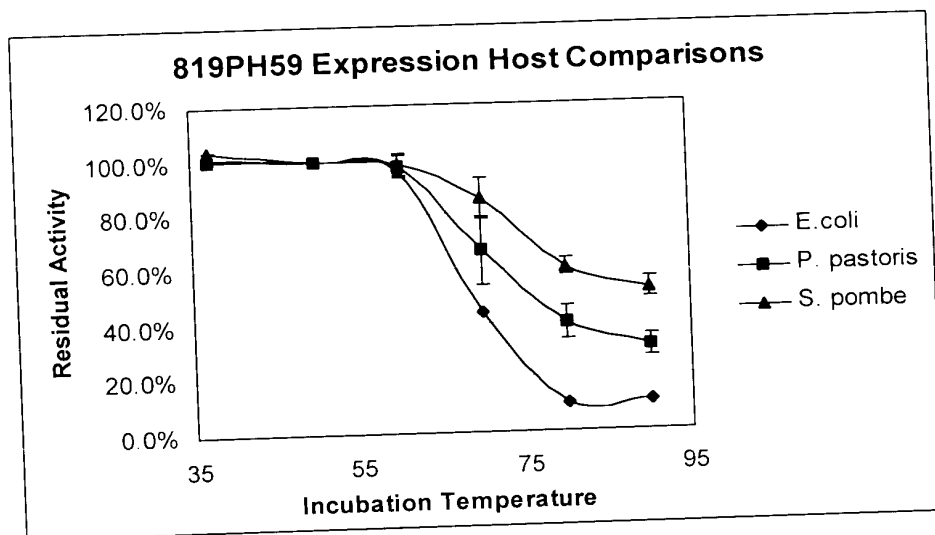


Figure 6.

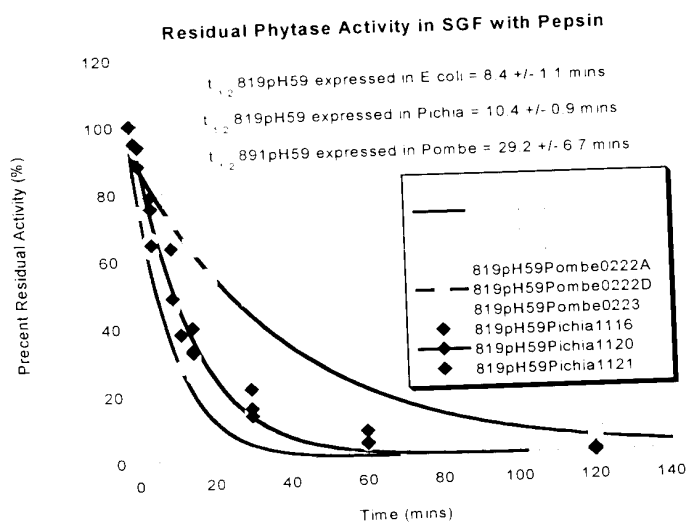


Figure 7a  
E. coli appA (GenBank accession no. M58708 ) (SEQ ID NO:7)

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg  
61 ctctccacc ttgtgttggt atggetggac ccgctctga aaagttaacg aacgtaggcc  
121 tgatgcggcg cattagcgc gcatcaggca atcaataatg tcagatatga aaagcgga  
181 catatcgatg aaagcgatct taatcccat tttatctct ctgattccgt taaccccgca  
241 atctgcattc gctcagagt agccggagct gaagctggaa agtgtggtga ttgtcagtcg  
301 tcatggtgtg cgtgtccaa ccaaggccac gcaactgatg caggatgtca cccagacgc  
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cngngtgggt agctaategc  
421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaanaaggg  
481 ctgcccgcag tctggtcagg tcgcgattat tgcgtatgtc gacgagcgta cccgtaaac  
541 aggcgaagcc ttcgccgcg ggctggcacc tgactgtgca ataaccgtac ataccaggc  
601 agatacgtc agtcccgatc cgttatttaa tctctaaaa actggcggtt gccactgga  
661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgactttac  
721 cgggcacggg caaacggcgt ttcgcgaact ggaacgggtg ctttaatttc cgcaatcaaa  
781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc  
841 ggaactcaag gtgagcgccg acaatgtctc attaacgggt gcgtaagcc tcgcatcaat  
901 gctgacggag atatttctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag  
961 gateaccgat tcacaccagt ggaacacctt gctaagtgtg cataacgcgc aattttatt  
1021 gctacaagc acgccagagg ttgccgcag ccgcgccacc ccgttattag atttgatcaa  
1081 gacagcgtt acgccccatc caccgcaaaa acaggcgat ggtgtgacat taccacttc  
1141 agtgcgttt atcgccggac acgatactaa tctggcaaat ctggcgccg cactggagct  
1201 caactggacg ctccccgtc agccggataa cacgccgcca ggtggtgaac ttgtgttga  
1261 acgtggcgt cggctaagcg ataacagcca gtggattcag gtttcgctgg tctccagac  
1321 ttacagcag atgcgtgata aaacgccgct gtcattaaat acgccgccg gagagggtga  
1381 actgacctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgttcgt tggcaggtt  
1441 tacgcaaat gtgaatgaag cagcatacc ggcgtgcagt ttgtaatgca taaaaagag  
1501 cattcagta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga  
1561 ccggaagagg cgttacgcc gcatccggc acttcagtt ttcctcttc tggagtaac  
1621 tataaccgta atagttag ccgtaactgt aagcgggtgt ggcgcgttta atcacacat  
1681 tgaggatagc gcccttaata ttgacgctg cctgttccag acgctgcatt gacaaactca  
1741 cctctttggc ggtgttcaag ccaaacgcg caaccagcag gctggtgcca acagaagcc  
1801 ccacgacgc ggcatactc accgccagca tcggcgccgt atcgacaac accagatcgt  
1861 aatggtcgtt cgcctatcc agtaattgac gcatccgatc g



Figure 7b

1 taaggagcag aaacaatgtg gtatttactt tgggtcgtcg gcattttgtt gatgtgttcg  
 61 ctctccaccc ttgtgttggg atggctggac ccgcgtctga aaagttaacg aacgtaggcc  
 121 tgatgcggcg cattagcate gcatcaggca atcaataatg tcagatatga aaagcggaaa  
 181 catatcgatg aaagegatct taateccatt ttatctctt ctgattccgt taaccccgca  
 241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg  
 301 tcattggtgtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca cccagacgc  
 361 atggccaacc tggccggtaa aactgggttg gctgacaccg cngngtggg agctaategc  
 421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaaggg  
 481 ctgcccgcag **tctgg**tcagg tccgcattat tgctgatgtc gacgagcgta cccgtaaaa  
 541 aggcgaagcc ttcgcccggc ggtggcacc tgactgtgca ataaccgtac ataccaggc  
 601 agatacgtcc agtcccgatc cgttatitaa tctctaaaa actggcggtt gccaaactgga  
 661 taacgcgaac gtgactgacg cgtatctcag cagggcagga gggtaattg ctgacttac  
 721 cgggcacggg caaacggcgt ttcgcgaact ggaacgggtg cttaatitc cgaatcaaa  
 781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc  
 841 ggaactcaag gtgagcggcg acaatgtctc attaacgggt gcggttaagc tgcacatca  
 901 gctgacggag atattctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag  
 961 gateaccgat tcacaccagt ggaacacett gctaagttg cataacgcgc aattttatt  
 1021 gctacaacgc acgccagagg ttcccgcag ccgcgccacc ccgttattg atttgatcaa  
 1081 gacagcgttg acgcccac caccgcaaaa acaggcgtat ggtgtgacat taccacttc  
 1141 agtgcgtttt atcgcgggac acgatactaa tctggcaaat ctggcgggcg cactggagct  
 1201 caactggacg ctcccgggtc agccggataa cacgcgcgca ggtggtgaac ttgtgtttga  
 1261 acgtggcgt cggctaagcg ataacagcca gtggattcag gtttcgctgg tcttcagac  
 1321 ttacagcag atgcgtgata aaacgcgct gtcattaaat acgcgcgccg gagaggtgaa  
 1381 actgaccctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgttcgt tggcaggtt  
 1441 tacgcaaatc gtgaatgaag cacgcatacc ggcgtgcagt ttgtaatgca taaaaaagag  
 1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga  
 1561 ccggaaaagg cgttcagcc gcatcggcc acttccagt ttctctttc tcggagtaac  
 1621 tataaccgta atagttag ccgtaactgt aagcgggtgt ggcgcgttta atcacaccat  
 1681 tgaggatagc gcccttaata ttgacgctg cctgttccag acgtgcatt gacaaactca  
 1741 cctctttggc ggtgttcaag ccaaacgcg caaccagcag gctgggtgcca acagaacgcc  
 1801 ccaagaccgc ggcacactc accgcagca tggcgggcgt atcgacaatc accagatcgt  
 1861 aatggtcgtt cggccattcc agtaattgac gcatccgac g

Figure 8  
Amino acid sequence for E. coli appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT  
PDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQV  
AIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLD  
NA  
NVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLT  
QALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQW  
NTLLSLHNAQFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTV  
LFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIQ  
VSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVN  
EARIPACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme  
(SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT  
PDAWPTWPVKLGELTPRGGELIAYLGHYWRQRLVADGLLPKCGCPQSGQVA  
IIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDN  
A  
NVTDAILEAGGSIADFTGHYQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ  
ALPSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWN  
TLLSLHNAQFDLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLF  
IAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVS  
LVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEA  
RIPACSL